CARNEGIE INSTITUTION OF WASHINGTON
DEPARTMENT OF GENETICS
COLD SPRING HARBOR, LONG ISLAND, N. Y.

January 20, 1950

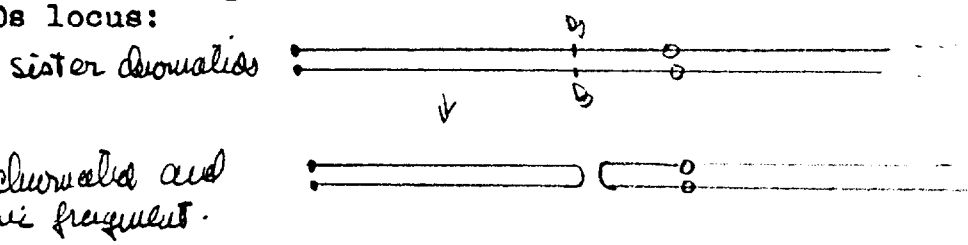
Dear Charlie,

Your good letter of January 4 was not answered before this as I have been trying to consider just how to get the information to you in order to answer the questions raised in your letter. It pleased me to learn that you had acquired so much about the Ds, Ac story when it was presented to you in such a rush and with no time for adequate annotation where necessary. I~~t~~ dont like to give you a short account that would be inadequate for a real comprehension; neither would I wish to burden you with the long accounts that have been written. As mentioned to you, these accounts were written, and are still being written, in order to get the data together and to have the correlations indicated in the required sequences. The accounts have been sent to Marcus regularly for he has been following the story with considerable interest since before he left for Illinois. He has also received samples of the kernels that illustrate the story. It seems to me that it would be wise for you to have samples of the various types of behavior if you are to grasp the descriptions with the least amount of confusion. Suppose, then, that I collect a group of such illustrations and send them on to you. If, after examining them and reading the descriptions that will be sent with each sample, you would like to see the full account, I should be glad to send on copies. I warn you, however, it is not easy reading, not because the subject is difficult to grasp but because of the number of experiments that have been made. It is a long chore and I would well understand the emotional reaction of not wanting to start such a task. If you could have stayed over for several days, we could have gone over the material here in short order and clarified the details quidkly. Now for your questions, however, or the comments.

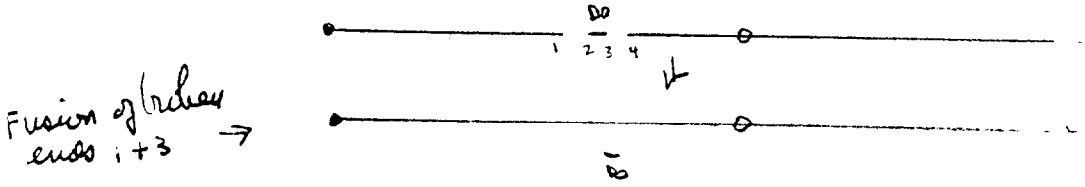
Comment 1. "The Ds locus was, when first located, on the short arm of 9 just outside the region with heavy chromomeres." This is correct. It lies to the right of Wx~~m~~ about 2 to 3 cross-over units away. This is called the standard location because it can appear at other locations.

Comment 2. This comment is complex but involves the mechanism of dicentric formation. Several types of events occur at the Ds locus as the consequence of ~~an~~ Ac initiated mutations. The frequency of one particular type of event depends on the "state" of the locus. It might be better worded to state that the "state" of a locus is reflected in the frequency of the particular types of events that can occur at the Ds locus. One of these events is the formation of an acentric fragment and a dicentric chromatid. This

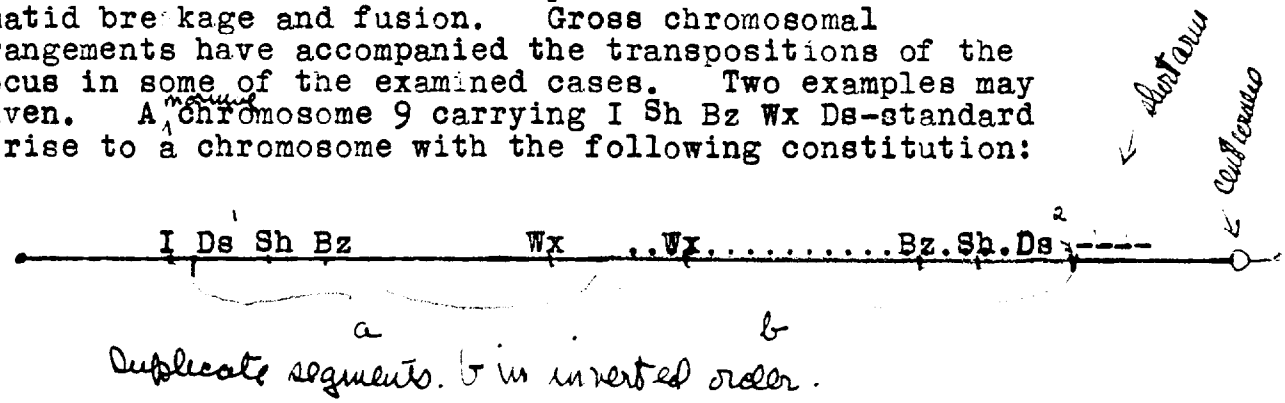
occurs at a mitosis -- probably during or just after division of the chromosome -- and involves both chromatids of chromosome 9. The positions where the fusions to give the acentric fragment and the dicentric chromatid occur at the Ds locus:



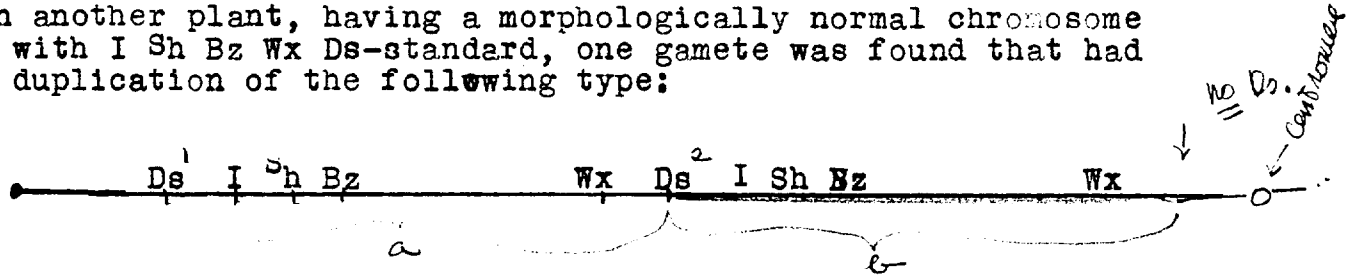
A second type of consequence of a Ds mutation results in the loss of the Ds locus from at least one of the chromatids of chromosome 9. The mechanism of this loss is not known but is inferred from the other events that are known to occur. It is possibly pulled out of the chromosome:



A third consequence of a mutational event at Ds results in the transposition of the Ds locus from one position to another. The mechanism of transposition is believed to be chromatid breakage and fusion. Gross chromosomal rearrangements have accompanied the transpositions of the Ds locus in some of the examined cases. Two examples may be given. A mutant chromosome 9 carrying I Sh Bz Wx Ds-standard gave rise to a chromosome with the following constitution:

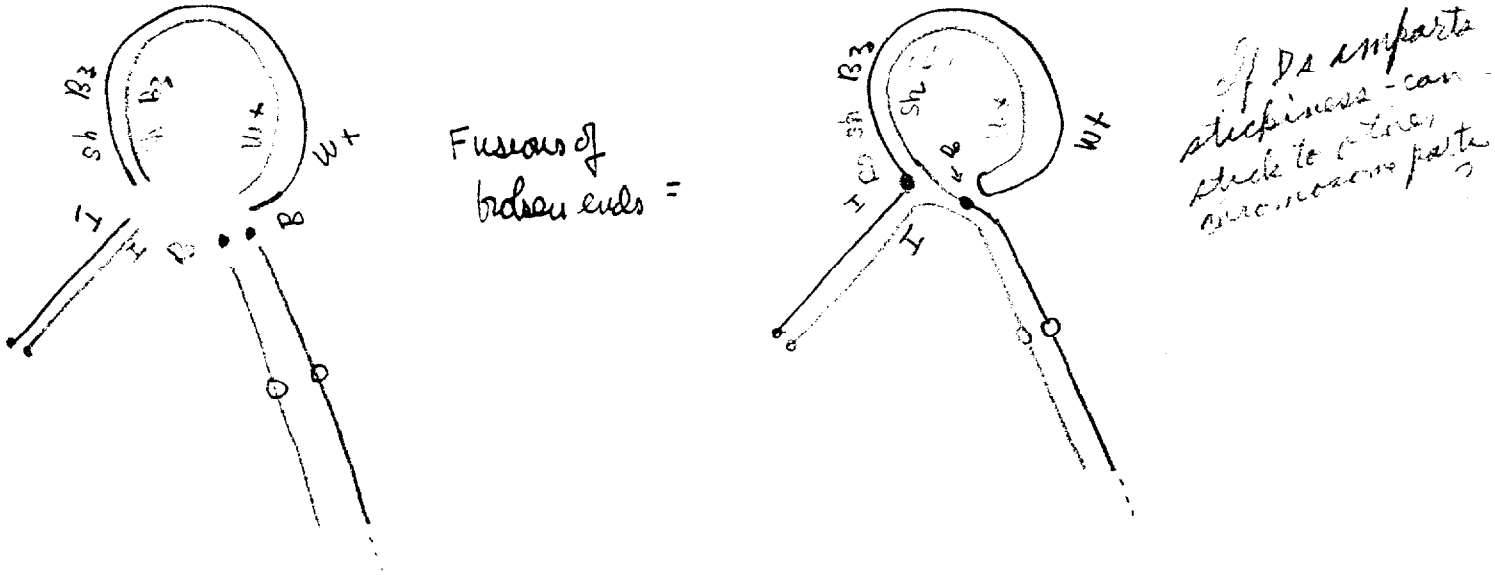


In another plant, having a morphologically normal chromosome 9 with I Sh Bz Wx Ds-standard, one gamete was found that had a duplication of the following type:

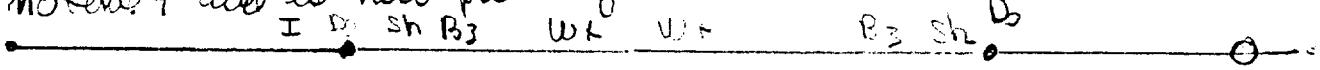


In both cases, two chromosome breaks (involving both chromatids at the same locus) are necessary to produce the duplications. It may be noted that one break occured at the known position of Ds and the other break occurred at a position which is marked by the new position or positions of Ds. That Ds events are associated with chromosome breakage and fusions is indicated by the usual consequence of Ds events -- dicentric chromatid formation. That the transpositions of Ds are associated with chromosome breakages that result in the insertion of Ds between broken ends could be suspected from the frequent association of transpositions with gross chromosomal rearrangements where the

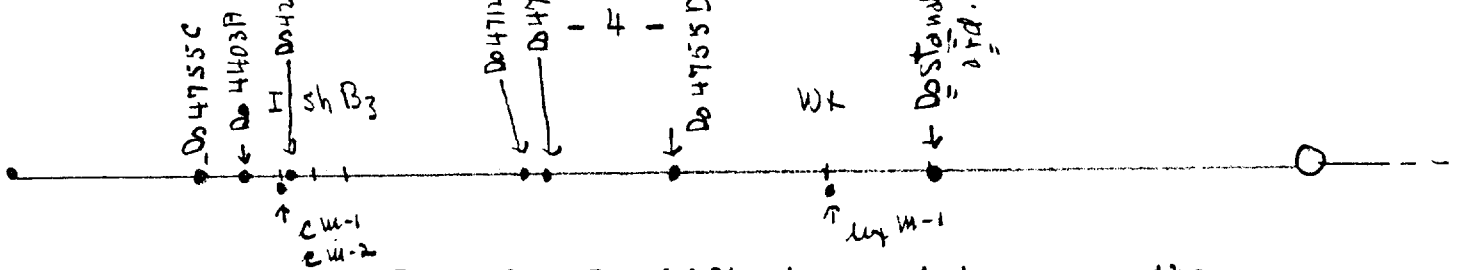
breakage positions can be well established, as in the above two cases. Either Ds was inserted as the consequence of a break that occurred elsewhere in chromosome 9 or Ds activity appeared at this new position as well as remaining at its standard location and the gross chromosomal rearrangement resulted from coincident Ds events at these two positions. The latter assumption is unlikely because, where these gross chromosomal abnormalities have arisen in association with a new location of Ds activity, no evidence of any sector having a normal chromosome 9 with Ds at the new position has been seen. Either one event must follow the other immediately or the appearance of Ds in a new position and the production of a gross chromosomal rearrangement are the consequences of a single event. Such an event may be diagrammed as follows:



Resulting chromosome = Transposed Ds and a Duplication of a segment in the inverted order with the position of the breaks marked by the former position of Ds in a normal chromosome 9 and the new position of Ds:



If the above is the mechanism responsible for transpositions of Ds, then only certain kinds of transpositions will be obtained from male gametes that may be recognized immediately on crossing to C sh bz wx ds female plants. The chromatids with long deficiencies will be eliminated. The translocations will not be recognized, Many of the duplications will not pass through the pollen because of the extent of the duplication. Also, normal chromosomes 9 with a Ds locus in a new position and no Ds ~~xxxxxxxx~~ locus in the old position or a morphologically normal chromosome 9 with two Ds loci, one at the new position and one at the old position will be found. Examples of the latter two types have been found, as expected. As far as the evidence shows, there is no position in chromosome 9 where Ds activity is liable to appear as the consequence of trans position. The various positions given below have been found so far and more are being investigated:



Comment 3. "When Ds shifts to a point near another gene it causes it to mutate, e.g. CDs = c; amd if Ds is subsequently lost, a reversion mutation occurs from c to C."

This is essentially correct. In the plants having Ds and Ac, new mutable loci are arising that respond to Ac as does Ds. One of these has received considerable study. This is the mutable locus called c-ml. The designation "m-1" refers to the first of the mutable c loci that were found. It appeared on the ear in a single kernel from the cross of a c sh wx ds ac female by a male plant having two morphologically normal chromosomes 9 carrying C Sh wx Ds in each chromosome. The Ds locus in both chromosomes was at the standard location. The C locus was normal in both chromosomes. It has been in my stocks for years and has always behaved as expected. In the cross of this plant to the c sh wx plants, nearly 3,000 kernels were obtained but only in a single kernel on the ear of one of these crosses did a peculiar type of behavior occur. Insead of showing colorless areas in a colored background, due to losses of the C locus following Ds mutations at the standard location, this kernel showed C spots in a colorless background. It was just the reverse of the expected type of variegation. It is from this kernel that the c-ml case originated. It proved to be as follows. A change from a normal C locus to a c locus capable of mutating back to C occurred coincidently with the disappearance of the Ds locus at the standard position and its appearance at a location that could not be separated from the mutable C locus. When this c mutates to C, all Ds activity ceases, except when Ds is transposed to another position coincident with the appearance of the C phenotype. The C locus, following a c-ml mutation, is just as normal as it was before the appearance of Ds activity at this position -- that is, before c-ml arose.

Comment 4. "In order that these mutations occur, an activator (Ac) must also be present. Here are some of the questions:

a) If the activator is not present (i,e,ac), does Ds break loose and shift?"

The answer to this is sharp. Ds will undergo breakage events only when Ac is present. If Ac is not also present in the complement, there is no evidence whatsoever of a Ds locus. To illustrate, if a chromosome has C Sh wx Ds, or I Sh Bz Wx Ds and is crossed to c sh wx or C sh bz wx, and if no Ac locus is involved, the kernel types are completely normal. There is no indication of any abnormal locus in these chromosomes. As soon as Ac is added, however, the presence of Ds will be apparent. Again, a c-ml locus in an ac constitution will be completely colorless and no mutations to C will occur.

Question (b) of this comment: "Your c-m1 and c-m2, are these similar in phenotype, but separate occurrences; or do they differ in phenotype?"

The answer is simple in only one aspect -- they differ decidedly. The c-m1 mutable locus mutates directly to a full C phenotype with few exceptions. c-m2 mutates to two different basic phenotypes, each of which may be expressed quantitatively. The two mutable c loci arose from normal C loci, but quite independently of one another but in plants having Ds and Ac loci.

Question (c) of this comment: "Is the Ds locus recognized cytologically after it is broken loose?"

The answer to this one is also simple. One cannot recognize any physical basis for a Ds locus. When it is transposed to a new position, such as between I and Sh, it does not interfer with crossing-over between these two loci. Recognition in a new position follows from the Ds breakage events that occur in this position. To illustrate: If Ds is at the standard location in an I Sh Bz Wx chromosome, the position will be to the right of Wx. When a Ds event occurs at this position, that gives rise to a dicentric chromatid and an acentric fragment, the marked loci I Sh Bz Wx will be carried by the acentric fragment. This fragment will be lost to the cell. All the cells arising from this cell will have no I Sh Bz Wx loci in endosperms that received this chromosome from the pollen and a C sh bz wx ds chromosome from the egg parent. A sector would appear in the kernel showing the C sh bz wx phenotype. If, however, Ds were located between Bz and Wx, the acentric fragment would carry I Sh Bz, but Wx would remain in the dicentric chromatid. The sector formed after a dicentric forming Ds event would be C sh bz but would be variegated for Wx to wx because of the breakage-fusion-bridge cycle initiated by the Ds breakage event. The position of these breaks may be seen in the microsporocytes. They coincide with the positions inferred from the genetic evidence (either linkage data or sector type or both). Thus, the Ds locus can not be seen but its position is determined by linkage, by breakage events observed in the endosperm or plant and by the breaks observed cytologically in the microsporocytes.

Comment 5. "Ac may shift into #9 or in another chromosome, Where was it when first located?"

The position of Ac when first discovered is not known. That it may go from one chromosome to another is apparent. It may go from one unknown chromosome to chromosome 9 and there be identified because of linkage with the marked loci on chromosome 9. After it has come into a position in chromosome 9, it may move off and into another chromosome. Over 50 transpositions of Ac have been observed, or better, investigated. Three of these entered chromosome 9, two in the long arm and one in the short arm.

Comment 6. This is related to what happens when two Ds loci are present. The answer to this depends upon several considerations. First, if the Ds loci are in different chromosomes - that is in homologoues and at the same location in each homologue. If the two Ds loci have the same frequency of the consequence of the Ds event a# number of results can occur depending on the states of these Ds loci. If the majority of the events give dicentric chromatids, coincident mutational events can be high and much homozygous deficient cell# production results. If one Ds locus has a high rate of dicentric formation as the consequence of the Ds event and the other a low rate of dicentric formation but a high rate of Ds loss, then the result is quite different, as can be pictured. In this latter case, the results are quite nice when the Ds loci in the two homologues do not occupy allelic positions. The case of c-ml and Ds-standard can be used to illustrate this If a plant having c-ml (Ds at the C locus to give a c phenotype) and wx is crossed by a plant having c (regular recessive allele, non-mutable with Ac) and W# and Ds-standard the folbowing constitution is present in the endosperm:

c-ml wx ds (2 of these chromosomes but the results given below are the same)

c Wx Ds

If the Ds events occurring at the c-ml in this selection of Ds give rise mainly to c to C mutations and few dicentrics and if the Ds in the c Wx Ds chromosome gives mainly dicentrics and few losses of Ds, coincident mutations are readily observed. A mutation of c-ml to C will give a cell have the normal C phenotype. The sector produced after such an event will be C. If a coincident mutation occurs at the Ds locus in the c Wx Ds chromosome, a dicentric chromatid and an acentric fragment will be formed. The acentric fragment will carry the Wx locus. Loss of this fragment will result in a sector with no Wx. It will be wx in phenotype. Coincident mutations of c-ml and Ds at the standard location would give sectors that are C and the underlying starch should be wx. The borders of the C and wx areas should coincide. That is, a C sector should have underlying wx starch. When the combination is made, as given above, just such sectors are very frequent.

You mentioned something about the sticking together of Ds loci -- the more loci, the more sticking. This was mentioned in our conversation because of the following observations. The Ds locus is able to change from one "state" to another "state". As mentioned above, the states of the Ds loci are identified by the frequency of alternate ~~events~~ consequences that can occur ~~as the consequence of~~ from a mutational event occurring at the Ds locus. Ds loci that are in a state giving dicentric chromatid formation as the consequence of the majority of the events, can change, by a single event, to a state that gives few dicentric events and many losses of the Ds locus without noticable alterations in the chromosome.

On the other hand, a Ds locus can go from one giving many dicentrics to one giving very few dicentrics in a single event.

A Ds locus in this latter state does not return to one giving a high frequency of dicentric chromatids by a single event. A sequence of events must take place, each event increasing the frequency of dicentric chromatid formation. It is this observations that has led me to suspect that the Ds loci giving high rates of dicentric formation have more Ds units than those giving low rates of dicentric formation on the assumption that it is easier to loose Ds loci by the mechanism associated with Ds breakage events than to gain them.

As you can imagine from the above brief discussion of the Ds Ac story, it is a very large one. Extensive examinations of a number of aspects of this story have been conducted. I have told you very little about Ac itself. Even though the details are manifold, obviously, there is a consistency that does not fail. You can see why I have not dared publish an acount of this story. There is so much that is completely new and the implications are so suggestive of an altered concept of gene mutation that I have not wanted to make any statements until the evidence was conclusive enough to make me confident of the validity of the concepts. I feel that I have made considerable progress and much that formerly was confusing is now quite sharp and clear. The size of the job ahead is staggering to contemplate, however. I dont know just what to do about it. So much needs to be done that only selections of the most important aspects can be considered. This involves a constant digest and reevaluation of the requirements.

Thank you for sending me the extra sheets of the manuscript. The past three weeks have been filled from early morning until late at night with no breaks. I shall have the required time this coming week-end and will send your copy with comments sometime next week.

Sincerely,

Bert

(concept Ds locus!)